Individual differences in autistic traits are associated with serotonin transporter gene polymorphism through medial prefrontal function: a study using NIRS

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INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder whose core symptoms include impairments or peculiarities in social interaction and communication (Volkmar and Pauls, 2003). Patients with ASD usually have abnormalities in brain function or structure (Ha et al., 2015). The diagnosis of ASD is based solely on behavioral characteristics (Volkmar and Pauls, 2003), with no objective criteria such as results on a blood test. Symptoms and their severity differ considerably among patients generically diagnosed as “ASD”.

Autistic traits are also seen in the general population, even in people for whom a diagnosis of ASD is not warranted. Indeed, autistic traits are distributed along a continuum, independent of the diagnosis (Constantino and Todd, 2003; Posserud, 2006) and there is no clear border between those who have autistic traits and who do not. ASD is located at the extreme end of the distribution. Further, evidence suggests that some genetic factors that affect

autistic traits are shared by ASD and subclinical populations (Constantino and Pauls, 2003; Mosconi et al., 2010; Robinson et al., 2011).

Genetic factors are thought to play an important role in the etiology of ASD (Bailey et al., 1995; Vorstman et al., 2006), but recently its association with certain early environmental factors has also become evident (Hallmayer et al., 2011). Because the behavioral characteristics of ASD result from multi-factorial heritability and interactions with environmental factors, individual differences in symptoms and their severity are considerable. This makes understanding the pathogenesis of ASD complicated.

Recently, gene polymorphism has drawn interest as a likely contributor to the differences in individual symptoms and severity found in ASD. In particular, serotonin transporter-linked polymorphic region (5-HTTLPR) has been investigated by several groups for association with ASD (Christine and Susan, 2008; Cook et al., 1997; Devlin et al., 2005; Kistner-Griffin et al., 2011; Klauck et al., 1997; Yirmiya et al., 2001). 5-HTTLPR comprises two variants, a low expressing short (S)-allele and a high expressing long (L)-allele, which result in altered transcriptional activity and function of the serotonin transporter (5-HTT) (Heils et al., 1996). 5-HTT is a key regulator of serotonergic neurotransmission (Canli et Lesch, 2007) and is the presumed site of action for selective serotonin reuptake inhibitors (SSRI). The serotonergic system is of interest in the etiology and pathogenesis of autism for several reasons: (1) ASD is associated with elevated whole blood serotonin levels (Cook end Leventhal, 1996; Dubravka et al., 2007; Lam et al., 2006); (2) core ASD symptoms can sometimes be improved by treatment with SSRIs (Dove et al, 2012; Gordon et al., 1993; Hollander et al., 2005; Hollander et al., 2012; Kolevzon et al., 2006; McDougle et al., 1996); (3) the brain serotonergic system is disrupted in patients with ASD (Chandana et al., 2005) and in animal models of autism

(Tamada et al., 2010). Serotonin is therefore a strong candidate molecule for influencing ASD and 5-HTTLPR is a potential candidate gene polymorphism linked to ASD. However, current findings are inconsistent, with different alleles or no alleles reportedly being associated with risk of ASD (Klauck, 2006).

In the current study, we assessed the effect of 5-HTTLPR on the wide range of autistic traits seen in the general population. By interposing an objective index of brain function between autistic traits and 5-HTTLPR, we were able to examine the possibility that 5-HTTLPR has an indirect effect on traits through mediation by brain function. We focused on the medial prefrontal cortex (mPFC) because previous studies have reported that it is associated with both ASD and the serotonergic system. The mPFC plays a role in social cognition (Grossmann, 2013), and altered mPFC activity in ASD has been reported to be associated with deficits in social communication and interaction ability (Ohnishi et al., 2000), including poor processing of facial affect (Harms, 2010; Watanabe et al., 2012). A study using near-infrared spectroscopy (NIRS) has reported that the degree to which healthy people exhibit autistic traits is correlated with how active the prefrontal cortex (PFC) is when people view images of facial expressions (Hosokawa et al., 2014).

Studies also indicate that mPFC function can be affected by serotonin or 5-HTTLPR. Indeed, serotonin is a major modulator of the PFC (Puig and Gulledge, 2011; Victoria) and plays an essential role in PFC function (Challis and Berton, 2015). Different 5-HTTLPR genotypes result in functional and anatomical differences in brain regions, including the mPFC (Jasinska et al., 2012; Rao et al., 2007), which might be related to why S-allele carriers exhibit higher sensitivity to social and emotional cues (Friedel et al., 2009; Heinz et al., 2005).

Additionally, altered 5-HTT binding capacity has been observed in the medial frontal area of individuals with ASD (Makkonen et al., 2008).

In light of these reported links between 5-HTTLPR, mPFC function, and ASD or autistic traits, we considered that the degree of mPFC activity during a social-emotional cognition task could be a relevant factor linking autistic traits and 5-HTTLPR. We hypothesize that 5-HTTLPR indirectly affects autistic traits via mediation by mPFC function. To date, no studies have examined the relationships between 5-HTTLPR, mPFC activation, and autistic traits in the same participants.

In this study, we evaluated mPFC function using a facial affect-labeling task that was based on the task in Nishikawa et al. (2015). Individuals with ASD have difficulty recognizing facial affect (Kasari et al., 1993; Lozier et al., 2014; Sigman et al., 1992), and children with autistic-like social communication difficulties have been reported to have similar difficulties (Kothari et al., 2013). Although patients with ASD show poor performance on facial affect-labeling tasks (Bölte and Poustka, 2018), training with this type of task can effectively improve daily life skill in recognizing facial affect (Wakamatsu, 2014). Training by facial affect labeling has also been effective in patients with stroke as it increases prefrontal activation in response to facial recognition (Shibasaki and Yoshida, 2016). We predicted that those who have greater autistic traits will show lower prefrontal activation during the facial affect-labeling task. Moreover, according to previous findings, the serotonergic system is involved in processing facial affect; scores for labeling facial affect increased in healthy participants who received SSRIs (Harmer et al., 2003) and the 5-HTTLPR genotype affects the ability to recognize facial emotion (Antypa et al., 2003).

This study comprises two experiments. Experiment 1 tested whether mPFC activation differs between those who have ASD and those who do not. In Experiment 2, we used the same task to examine the relationship between 5-HTTLPR, mPFC activation, and autistic traits in sub-clinical volunteers. Clarifying the neurogenetic basis of autistic traits in the sub-clinical population may help us do the same for ASD. Thus, we determined whether individual levels of autistic traits are indirectly affected by 5-HTTLPR through mediation by mPFC function.

EXPERIMENTAL PROCEDURES

Experimental design

Experiment 1: The aim was to examine whether mPFC activation induced by the facial affect-labeling task differs between those who have ASD and those who do not. We measured mPFC activity levels using NIRS and assessed levels of autistic traits using the Autism-Spectrum Quotient (AQ). These measures were compared between the ASD group and a typical development (TD) group.

Experiment 2: The aim was to determine whether individual autistic trait levels were associated with 5-HTTLPR in those without ASD, and if so, whether the relationship was mediated by neural activity involved in social functions. In addition to the measures used in Experiment 1, participants were genotyped for 5-HTTLPR. The correlation and mediation analyses were conducted using Structural Equation Models (SEM) among AQ scores, changes in mPFC activation, and 5-HTTLPR genotype.

Participants

All participants were right-handed. Written informed consent was obtained for each experiment; from all parents before Experiment 1 and from all participants before Experiment 2. The study was performed in accordance with the declaration of Helsinki and approved by the Ethics Committee of Clinical Study, Hiroshima University Hospital.

Experiment 1: Twenty children participated (ASD: n = 9, 8 boys and 1 girl, aged 7–14 years; age- and sex-matched TD: n = 11, 7 boys and 4 girls, aged: 5–12 years). All children with ASD were recruited from the Department of Pediatrics, Hiroshima University and had been diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V). The intelligence quotients (IQs) of the children with ASD were measured using the Wechsler Intelligence Scale for Children, third or fourth edition, and all had a full-scale IQ above 70. TD children were recruited from the local community, and none were suspected of any developmental abnormalities. None of the TD children had siblings with ASD.

Experiment 2: One hundred eighty neurotypical Japanese adults participated (121 males, 59 females; mean age: 23.9 ± 2.8 years). None suffered from any psychiatric disease or were suspected of any developmental abnormalities. In order to investigate a wide range of individual differences, no restriction was made with respect to the AQ total score.

Autistic traits assessment

The Japanese version of the AQ was used to assess the level of autistic traits. In Experiment 1, the parents were asked to fill out the children's version of the AQ (Wakabayashi, 2007) and in Experiment 2 the participants completed the adult version (Wakabayashi et al., 2004) by themselves. The questionnaires consisted of 50 items, made up of five 10-item sub-domains

(social skills, attention switching, attention to detail, communication, and imagination). Each item is worth one point, making the maximum AQ total score equal to 50 (and that for each sub-domain equal to 10). Higher scores indicate higher levels of autistic traits.

Facial affect-labeling task

The facial affect-labelling task was based off the task in Nishikawa et al. (2015) and was designed to evaluate mPFC activation related to social/emotional cognitive processing (Fig. 1). The task was performed using a personal computer with a touch panel and consisted of pre-task, task, and post-task periods. In the task period, participants were requested to view a woman's face displayed on the monitor and use the touch screen to select a verbal label that best described how the woman was feeling. Five labels (rectangles) were displayed at the bottom of the screen, each with one of the following words: "happy", "sad", "angry", "surprised", and "I am not sure" (written in Japanese). Each of four affective faces (happy, sad, angry, and surprised) was presented twice in random order for a total of 8 trials. Each trial ended as soon as one of the labels was touched.

In the pre- and post-task periods, only neutral faces were displayed. The participants were instructed to look at the neutral face and select the label "Neutral" (written in Japanese). The other four labels were blank. The location of the "Neutral" label was randomly chosen for each trial. Each trial ended as soon as any label was touched.

The pre-task, task, and post-task periods included 6, 8, and 8 trials, respectively. Each period began with an instruction displayed for 3 seconds that informed the participants about the period. Trials were separated by a 1 second interval during which a blank black screen was presented. The affective and neutral face stimuli were the "averaged faces" used by Maki et al.

(2013). Images were made by synthesizing standardized photos of four Japanese women from database DB99 (Advanced Telecommunications Research Institute International, Inc. Nara, Japan) to exclude non-emotional confounding factors and individual features of expressing emotions.

NIRS measurement

A two-channel NIRS machine (NIRO-200NX; Hamamatsu Photonics, Hamamatsu City, Japan) was used to measure changes in oxygenated-hemoglobin (oxy-Hb) and deoxygenated-hemoglobin (deoxy-Hb) concentration in the bilateral mPFC. Two probes were symmetrically placed on both sides of the forehead surface, at Fp1 and Fp2, according to the international 10-20 system used in electroencephalography. This probe placement was chosen in reference to previous studies to detect the regional cortical oxygenation in mPFC (Okamoto et al., 2004). The NIRS machine used three different wavelengths of near-infrared light (735, 810, and 850 nm) and tracked the variation in oxy-Hb and deoxy-Hb concentrations that were calculated (via the Beer-Lambert law) from the changes in light absorption. The distance between the emission and detection probes was 3.0 cm. The NIRS machine measures changes in hemoglobin concentration approximately 2–3 cm beneath the surface of the skull, which only incudes the surface of the cortex. The time resolution of the NIRS signal was 1.0 second. Changes in oxy-Hb (ΔOxy-Hb) were used for the analysis because it is known to reflect cortical activity better than changes in deoxy-Hb (Hoshi et al., 2001). To compare mPFC activation among participants, we normalized the raw ΔOxy-Hb by converting it to a z-score at each NIRS channel: the mean ΔOxy-Hb from baseline (10 seconds just before the task period) through the task period (5–15 seconds) was divided by the standard deviation of the baseline ΔOxy-Hb.

Genotyping

Genotyping of 5-HTTLPR was performed in Experiment 2. Buccal cells were sampled using mouth swabs (GE Healthcare Japan), and genomic DNA was extracted using a QIAmp DNA Investigator Kit (Qiagen Inc, Tokyo, Japan). The SLC6A4 gene promoter region was amplified by polymerase chain reaction (PCR). The forward primer (5′-GGCGTTGCCGCTCTGAATGC-3′) and reverse primer sequences (5′-GAGGGACTGAGCTGGACAACCAC-3′) were the same as those previously described (Tomoda et al., 2013). The 50 µl reaction mixture contained 50 ng genomic DNA, 1.25 U of Tks Gflex DNA Polymerase (Takara Bio, Japan), 25 µl of 2 × Gflex PCR buffer (Takara Bio, Japan), and 15 pmol of each primer. The protocol for PCR was as follows: initial denaturation (94°C for 1 min) followed by 35 amplification cycles (denaturation at 98°C for 10 s, annealing at 60°C for 15 s, and extension at 68°C for 1 min). The PCR products were separated using electrophoresis in a 3% agarose gel stained with ethidium bromide and visualized by ultra-violet light. The observed 484 bp band indicated the short (S) allele and the 528 bp band indicate the long (L) allele. Each participant was identified as one of 3 groups: homozygote for the S-allele (S/S), heterozygote for the S- and L-alleles (S/L), and homozygote for the L-allele (L/L).

Statistical analysis

Experiment 1: Analyses were performed using IBM SPSS version 23 (IBM Corp., Armonk, NY, USA). Student's *t*-test were conducted for age, AQ score, and mean ΔOxy-Hb z-score in each channel between the ASD and TD groups, and Fisher's exact test was conducted for

gender. Correlation analyses were conducted between AQ scores and the mean ΔOxy-Hb z-score for each channel of each diagnostic group.

Experiment 2: A one-way ANOVA was performed for age, and Pearson's chi-square test (2-sided) was conducted for gender between the 5-HTTLPR genotypes to verify the inter-group variability. Correlation analyses were conducted among AQ scores, mean ΔOxy-Hb z-score in each channel, and the number of 5-HTTLPR L-alleles. Mediation analysis with Structural Equation Models (SEM) was performed using AMOS version 23 (an add-on to the SPSS statistical software) to test the 5-HTTLPR-mPFC-autistic traits pathway. We assessed the potential effect of mediation according to the "causal steps approach" described by Baron and Kenny (1986). In the first analysis, a direct path from independent variable X (5-HTTLPR) to dependent variable Y (AQ score) was drawn to analyze the direct relationship. In the second analysis, an indirect path through variable M (oxy-Hb) was added to the first path. If the direct effect from X to Y disappears or is strongly reduced in the second analysis, variable M can be interpreted as a significant mediator. Model fit was assessed by chi-square test (χ^2), the General Fit Index (GFI), the Comparative Fit Index (CFI), and the Root Mean Square Error of Approximation (RMSEA). Acceptable fitting models require nonsignificant chi-square test, GFI > 0.90, CFI > 0.90, and RMSEA < 0.08.

RESULTS

Experiment-1

Basic participant characteristics

Age and gender did not differ significantly between the TD and ASD groups (Table 1). There were no gender differences in age, ΔOxy-Hb, or AQ scores in either diagnostic group. Participant age was not correlated with the mean ΔOxy-Hb z-score or the AQ scores in either diagnostic group.

variables	ASD	TD	Group differences	
	(n=9)	(n=11)	*t*-value	*p*-value
Females (n, %)	1 (11.1)	4 (36.3)		0.32
Age	11.5 (1.8)	9.9 (3.0)	-1.4	0.18
AQ scores				
Total	27.8 (5.4)	13.5 (5.6)	-5.8	<0.01
Social skills	5.0 (2.4)	2.7 (1.6)	-2.6	0.02
Attention switching	6.2 (1.1)	3.5 (1.8)	-4.1	<0.01
Attention to detail	4.1 (1.7)	2.7 (1.2)	-2.1	0.046
Communication	5.9 (2.0)	2.2 (1.8)	-4.3	<0.01
Imagination	6.6 (2.1)	2.4 (1.6)	-5.2	<0.01

Table 1. Number of participants in each group and their mean age and AQ scores. Higher AQ scores indicate higher autistic traits. Age and AQ scores are presented as means (standard deviations).

AQ score

The ASD group had higher AQ total scores ($p < 0.01$; Fig. 2A) and higher sub-domain scores (social skills: $p = 0.02$; attention switching: $p < 0.01$; attention to detail: $p = 0.04$; communication: $p < 0.01$; imagination: $p < 0.01$) than TD group (Table 1).

NIRS data

The oxy-Hb (z-score) in the right and left mPFC during the task period (from 5 to 15 s) was significantly higher than baseline in the TD group (right mPFC: $p = 0.01$; left mPFC: $p < 0.01$) but not in the ASD group (right mPFC: $p = 0.1$; left mPFC: $p = 0.74$). The mean ΔOxy-Hb z-scores in bilateral mPFC were significantly lower in the ASD group than in the TD group (right mPFC: $p < 0.01$; left mPFC: $p = 0.01$; Fig. 2B). The association between mPFC activation and AQ scores was examined using Pearson's correlation coefficient for each diagnostic group and for all participants. Significant correlations were observed between several parameters (Table 2).

Parameters		Mean z-score for the change in oxy-Hb					
		ASD group		TD group		all participants	
		rmPFC	lmPFC	rmPFC	lmPFC	rmPFC	lmPFC
AQ scores	Total	-0.687*	-0.657*	-0.658	-0.682*	-0.819**	-0.762**
	Social skills	-0.668*	-0.625*	-0.642	-0.601	-0.727**	-0.698**
	Attention switching	-0.462	-0.337	-0.308	-0.332	-0.689**	-0.584**
	Attention to detail	0.446	0.379	-0.128	-0.605	-0.171	-0.312
	Communication	-0.693*	-0.282	-0.191	-0.193	-0.730**	-0.535*
	Imagination	-0.254	-0.039	-0.226	-0.377	-0.538**	-0.356

Table 2. Pearson's correlation analyses between the mean ΔOxy-Hb z-scores in the right and left mPFC and AQ scores for each diagnostic group and for all participants. The values represent the Pearson's r correlation coefficients. * $p < 0.05$, ** $p < 0.01$.

Experiment-2

Basic participant characteristics

We found no gender differences in age, ΔOxy-Hb, or AQ scores. We found no significant differences in gender or age among the three genotypes (Table 3). Participant age did not correlate with the mean ΔOxy-Hb z-scores or AQ scores. The frequency distributions of the 5-HTTLPR genotypes were as follows: S/S: 103, S/L: 67, L/L: 10. Observed genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium ($\chi^2 = 0.04$, $p = 0.76$).

variables	S/S genotype (n=103)	S/L genotype (n=67)	L/L genotype (n=10)	Group differences *p*-value
Females (n, %)	35 (34)	19 (28.4)	5 (50)	0.37
Age	23.8 (2.75)	24.0 (2.68)	24.5 (3.10)	0.65

Table 3. Number of participants in each group and their mean age. Ages are presented as means (standard deviations).

5-HTTLPR genotype effect on autistic traits and mPFC activation

Analysis using Spearman's rank correlation coefficients were used to examine whether the number of L-alleles was correlated with AQ scores or the mean ΔOxy-Hb z-score in the mPFC (Table 4). Analysis revealed significant correlations with scores on the AQ social skills

subdomain and with mean ΔOxy-Hb z-scores in right mPFC (ρ = 0.203 and ρ = 0.364 respectively, both $p < 0.01$).

Parameters		Number of L-allele
AQ scores	Total	0.125
	Social skills	0.203**
	Attention switching	0.004
	Attention to detail	0.035
	Communication	0.027
	Imagination	0.031
Mean z-score for the change in oxy-Hb	right mPFC	-0.364**
	left mPFC	-0.121

Table 4. Spearman's rank correlation analysis between the number of 5-HTTLPR L-alleles and other factors (AQ scores and mean ΔOxy-Hb z-scores for right and left mPFC). The values represent the Spearman's rho correlation coefficients. *$p < 0.05$, **$p < 0.01$.

Relationships between mPFC activation and AQ scores

Pearson's correlation coefficient was used to examine the association between mPFC activation and AQ scores. We found significant negative correlations between mean ΔOxy-Hb z-score in the right mPFC and AQ total score ($r = -0.271$, $p < 0.01$), AQ social skills score ($r = -0.395$, $p < 0.01$; Fig. 3), AQ attention-switching score ($r = -0.228$, $p < 0.01$) and AQ communication score ($r = -0.276$, $p < 0.01$) (Table 5).

Parameters		Mean z-score for the change in oxy-Hb	
		right mPFC	left mPFC
AQ scores	Total	-0.271**	-0.191*
	Social skills	-0.395**	-0.184*
	Attention switching	-0.228**	-0.150*
	Attention to detail	0.142	0.038
	Communication	-0.276**	-0.245**
	Imagination	-0.069	-0.057

Table 5. Pearson's correlation analysis between the mean ΔOxy-Hb z-scores in right and left mPFC and AQ scores. The values represent the Pearson's r correlation coefficients. *$p < 0.05$, **$p < 0.01$.

SEM evaluation

Based on the correlation analyses, we hypothesized that the right mPFC activation mediated the effect of 5-HTTLPR genotype on autistic traits related to social skills. We designed path models to test this hypothesis. We used the number of 5-HTTLPR L-alleles as the independent variable, AQ social skills score as the dependent variable, and the mean ΔOxy-Hb z-score in right mPFC as the mediator in the pathway. The first analysis examined the model that includes only a direct effect from 5-HTTLPR to AQ social skills score. This model was completely saturated and provided a perfect fit ($\chi^2 = 0$, df = 0, $p > 0.01$, CFI = 1.00, RMSEA = 0.00). The direct path was significant ($\beta = 0.22$, $p < 0.01$). Next, to assess the extent to which right mPFC activation reduced the magnitude of the direct effect, we analyzed a mediation model comprising the direct and indirect paths (Fig. 4A). This model was also completely saturated

and provided a perfect fit ($\chi^2 = 0$, df = 0, $p > 0.01$, CFI = 1.00, RMSEA = 0.00). The paths from 5-HTTLPR to right mPFC ($\beta = -0.33$) and from mPFC to AQ social skills ($\beta = -0.36$) were both significant ($p < 0.01$). However, the direct path from 5-HTTLPR to AQ social skills was not significant ($\beta = 0.097$, $p = 0.18$). The reduction was from $\beta = 0.22$ ($p < 0.01$) to $\beta = 0.097$ (ns). Thus, controlling for the mediating effect of right mPFC eliminated the direct effect. The model that only included the indirect path also had an acceptable fit ($\chi^2 = 1.79$, df = 1, $p = 0.18$, GFI = 0.99, CFI = 0.98, RMSEA = 0.066, see Fig. 4B). All pathways were significant in this model ($p < 0.01$).

DISCUSSION

In Experiment 1 we examined whether mPFC activation induced by the facial affect-labeling task differs between those who have ASD and those who do not. The NIRS results showed that the task normally activates bilateral mPFC—the region responsible for recognition of facial emotion (Heberiein et al., 2008)—and that activity levels were significantly lower in the ASD than in the TD group. Simultaneously, we confirmed that individuals who have higher AQ scores (i.e., the ASD group), show lower mPFC activity when engaged in the facial affect labeling task. The reduced mPFC activation that we observed in the ASD group can be considered to be associated with impaired ability to recognize facial emotion. Previous findings suggest that the role of the mPFC when viewing an affective face is to inhibit amygdala responses (Hariri et al., 2000; Lieberman et al., 2007), which then allows the emotion to be judged correctly (Heberiein et al., 2008). Indeed, patients with lesions confined to ventral mPFC have been reported to show impaired recognition of facial emotion (Heberlein et al.,

2008). Furthermore, when participants evaluate the emotion of an affective face and label it by putting the emotions into words, the induced activation in the amygdala is further suppressed by the mPFC (Hariri et al., 2000; Lieberman et al., 2007). The level of right PFC activity during facial affect labeling has been reported to be negatively correlated with activity levels in the amygdala, perhaps reflecting cognitive control of emotional responses through appraisal and evaluation of emotional stimuli (Hariri et al., 2000). This inhibitory function of the mPFC helps to alleviate emotional distress and plays an important role in emotion regulation (Etkin et al., 2012; Lieberman et al., 2007; Motzkin et al, 2015; Quirk and Beer, 2006). A previous study has reported that poorer ability to recognize facial emotion is correlated with deficits in social communication skills in children with ASD. This implies that the reduced mPFC activation that we saw in the ASD group might contribute to the dysfunction in how cognitive emotions are evaluated and underlie the social impairments of ASD. Therefore, we next examined the link between mPFC activation, the autistic traits, and 5-HTTLPR.

In Experiment 2 we investigated whether autistic traits were associated with 5-HTTLPR in sub-clinical participants, and if so, was the association mediated by neural activity related to social functions (i.e., the reduced mPFC activity observed in Experiment 1). AQ scores revealed that people with large numbers of 5-HTTLPR L-alleles have stronger autistic social skill traits. This result is supported by a previous study demonstrating that individuals with high 5-HTT-expressing L_A/L_A genotypes had lower social skills than those with low 5-HTT expressing S genotypes or who were L_G-allele carriers (Gadow et al., 2013). However, a previous study has reported that S-allele carriers with ASD show more severe social and communication deficits (Tordjman et al., 2001). We discuss this in more detail below.

More interestingly, we found that individuals carrying larger numbers of L-alleles

showed lower right mPFC activation in response to facial affect labeling. This tendency suggests weaker engagement of emotion-evaluation processes during facial affect labeling in these individuals. Our results are supported by previous studies showing that individuals with high 5-HTT-expressing L_A/L_A genotypes are less able to accurately recognize facial expressions of emotion (Boll and Gamer, 2014), and that the intensity threshold for recognizing negative facial expressions is higher for individuals with L/L genotypes than it is for S-allele carriers (Antypa et al., 2011).

More related to our hypothesis, correlation analysis showed that the right mPFC activity was negatively correlated with the AQ social skills score, which is correlated with the number of L-alleles. This result is consistent with the result observed in Experiment 1 in which lower mPFC activity was observed in the ASD group, which also had higher total AQ and AQ social skills subdomain scores. Together with Experiment 1, we have thus demonstrated the possibility that right mPFC activity engaged in facial affect recognition is the neural correlate of some autistic traits. Furthermore, our SEM analysis confirmed that right mPFC activation plays a mediator role in connecting 5-HTTLPR and autistic traits related to social skills. The mediating effect was not seen in the left mPFC. These results suggest a possible neural mechanism underlying the effect that 5-HTTLPR has on individual differences in social skills seen in ASD, and the possibility that the cognitive process involved in evaluating emotional stimuli can mediate the genotype-dependent tendency seen in these autistic traits. Thus, our findings offer evidence for genetic and neural correlates of autistic traits related to social skills.

Caution should be exerted when attempting to generalize the implication of our gene, neuroimaging, and phenotype results. Although our results showed that the S-allele was associated with lower autistic traits related to social skills, as mentioned above, another study

has reported that the S-allele is highly associated with deficits in sociability and communication (Tordjman S, et al., 2001). Furthermore, many previous studies have reported that the S-allele is linked to negative outcomes, such as increased anxiety-traits and risk of psychiatric disturbances in response to stressful life events (Caspi et al., 2003; Juhasz et al., 2015; Lesch et al., 1996). A possible explanation for this apparent discrepancy is that S-allele carriers tend to be more sensitive to environmental stimuli (Kiser et al., 2012). Homberg and Lesch have mentioned in their review that hypervigilance of S-allele carriers may be the common denominator in social cognitive superiority and anxiety-related traits, and that environmental conditions determine whether a response will turn out to be positive (cognitive, in conformity with the social group) or negative (emotional) (Homberg and Lesch, 2011). Under favorable environments such as supportive and enriching social conditions, S-allele carriers show adaptive outcomes, improved cognition, and social conformity. In contrast, in adverse environments such as distressing and oppressive social conditions, S-allele carriers tend to be emotional, leading to maladaptive responses and increased risk for mood disorders (Homberg and Lesch, 2011). Kochanska et al. (2011) have reported that while the social competence of L/L genotype carriers is not affected by early rearing environment, S-allele carriers reared by unresponsive mothers had significantly lower social competence compared to those reared by responsive mothers or to those with L/L genotypes. These findings indicate that the social competence of S-allele carriers is sensitive to individual experiences and environmental conditions. Taken together, we can say that higher neural activity in response to emotional stimuli and higher facial affect-recognition ability (both related to the S-allele) can lead to maladaptive social behavior, depending on experiences and environmental conditions.

Therefore, our observation does not necessarily imply that S-allele carriers always show

lower autistic traits related to social skills. Further studies are needed to understand whether the discrepancy between our results (a positive aspect of the S-allele) and those from previous studies (negative aspects of the S-allele) can be explained by the higher sensitivity of S-allele carriers. Furthermore, since the pathogenesis of ASD includes early negative environmental factors, the higher social skills observed in the sub-clinical S-allele carriers of this study is not necessarily identically expressed in patients with ASD. The discrepancy in results among studies that have examined the relationship between the S-allele and social skills may depend on individual participant characteristics, such as the ASD diagnosis or other environmental factors. Future research should separately examine ASD and healthy participants, considering the effects of environmental factors. Having said that, it is interesting to note that individuals who scored 9 or 10 on AQ social skills in our study were all L-allele carriers (Fig. 3).

This study has four methodological limitations, which are described below.

1. This study only included Japanese participants. Previous studies have shown differences in neural activation patterns and transcultural social behaviors among cultural groups (Kobayashi et al., 2006; Koelkebeck et al., 2011). Furthermore, ethnic difference in the influence of 5-HTTLPR on brain networks has also been reported (Kong et al., 2014). Replication studies in other ethnicities are needed to confirm our findings.
2. We did not consider gender difference because we could not obtain enough female participants with ASD in Experiment 1 (only one female). Previous studies have reported significant differences in ASD prevalence between males and females (Lai et al., 2014; Fombonne, 2003; Werling et al., 2013) and significant gender differences in clinical presentation, including social and communicative domains (Werling et al.,

2019; Kirkovski et al., 2013). This limitation means that the current findings should be interpreted cautiously.

3. All participants were right-handed. Previous research has reported that left-handers and mixed-handers are more common among people with ASD than among the general population (Rysstad et al., 2018). The current study reports the role of the right mPFC only in right-handed participants. Further research is needed to determine whether our results can be replicated in left-handers or mixed handers.
4. Despite the significant correlation of genotype and AQ social skills scores in Experiment 2, we must be cautious about inferring a gene-trait interaction because the sample size is small (Only 10 participants had L/L genotypes). Future studies are expected to confirm our findings using larger sample sizes.

In conclusion, the present study provided evidence of a link between 5-HTTLPR and the degree of autistic traits related to social skills. The neuroimaging findings indicate that higher autistic traits predict lower mPFC activation when trying to discern facial emotions. More interestingly, our analysis revealed a mediator role for the right mPFC in linking 5-HTTLPR and autistic traits related to social skills, suggesting a potential neural mechanism that controls how 5-HTTLPR effects individual autistic traits. Further studies of patients with ASD will provide additional biological evidence for the clinical features of ASD, and provide targets for potential pharmacotherapy and medical treatment.

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Figure Captions

Figure 1. Experimental protocol for the facial affect-labeling task. The actual labels were in Japanese.

Figure 2. (A) Distribution of AQ total score for TD and ASD. (B) Distribution of mean z-score of Δoxy-Hb in the left and right mPFC for TD and ASD. Solid horizontal lines represent the mean value. The mean values for ASD were both significantly different than those for TD ($p < 0.05$).

Figure 3. A scatter plot showing the relationship between AQ social skills score and mean ΔOxy-Hb z-score in the right mPFC of each participant. A negative correlation is visible, shown by the straight line that was obtained by a fit using all the data.

Figure 4. The mediating role of the right mPFC in the association between 5-HTTLPR and autistic traits related to social skills. (A) Mediation model illustrating how right mPFC activation mediates the direct effect between 5-HTTLPR and autistic traits related to social skills. The number in parenthesis under the path from 5-HTTLPR to the social skills is the coefficient for this direct path when the indirect path is not included. (B) Mediation model including only the indirect effect through right mPFC, eliminating the direct effect from 5-HTTLPR to autistic traits related to social skills. *$p < 0.05$, **$p < 0.01$, ns; not significant.

Figure 1

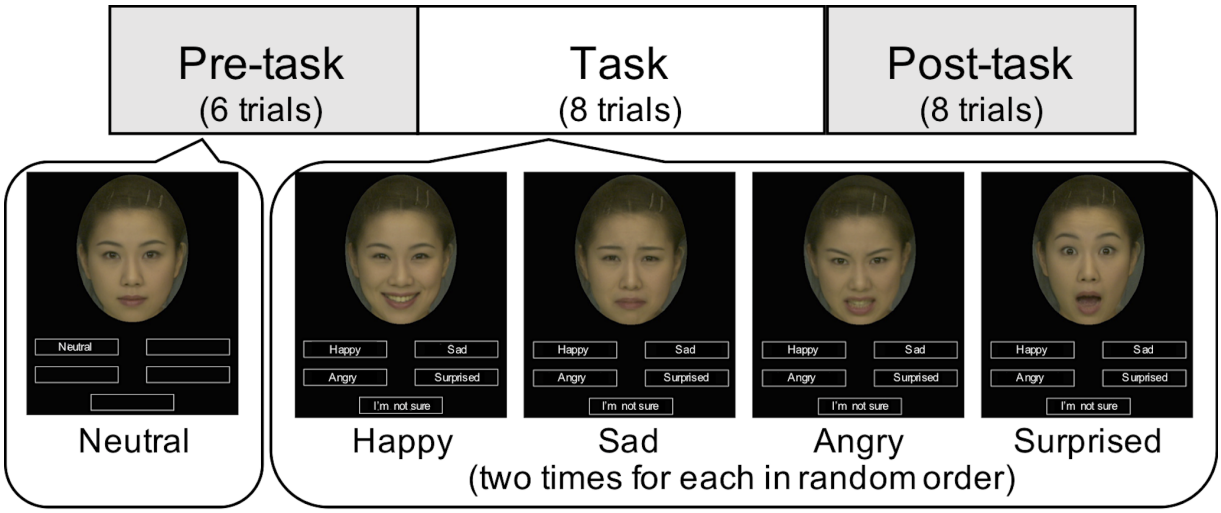

Figure 2

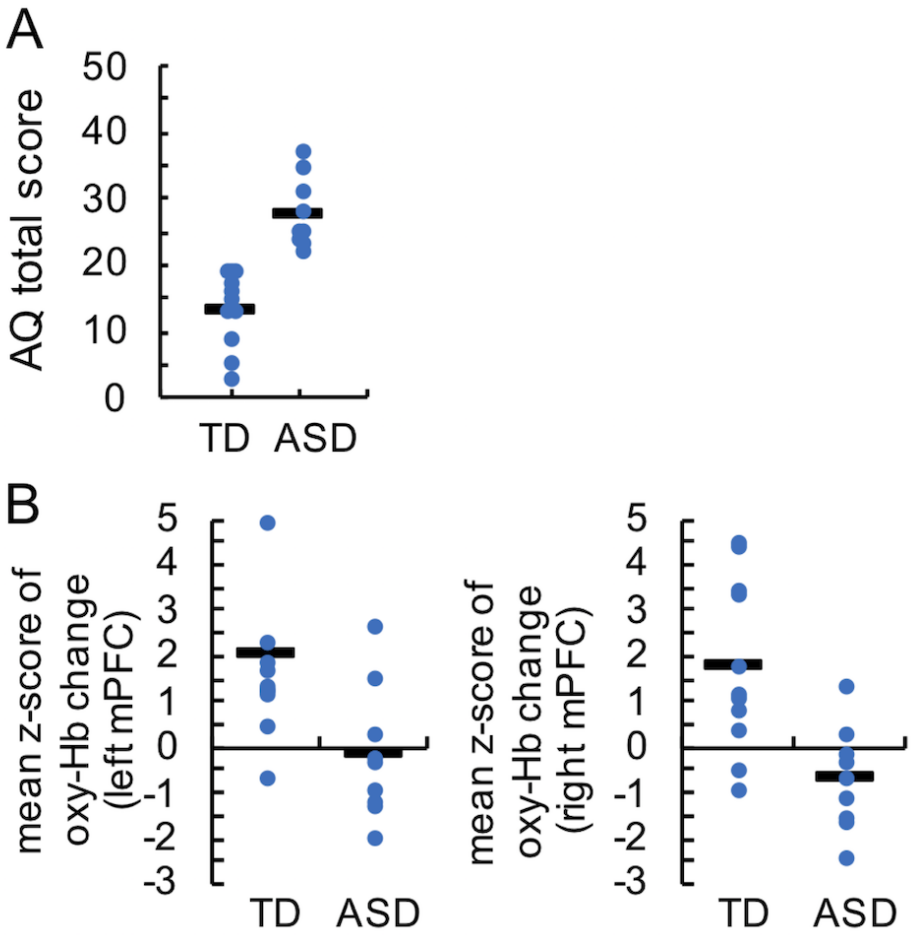

Figure 3

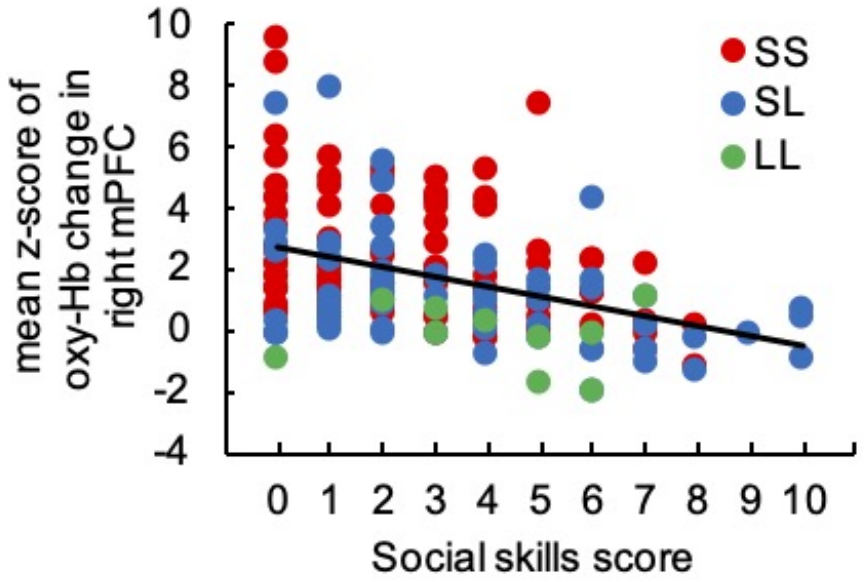

Figure 4